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## Topological and epitope mapping of the cellular retinaldehyde-binding protein from retina.

Crabb JW, Gaur VP, Garwin GG, Marx SV, Chapline C, Johnson CM, Saari JC.

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Cellular retinaldehyde-binding protein (CRALBP) carries 11-cis-retinol or 11-cis-retinaldehyde as endogenous ligands and may function as a substrate carrier protein that modulates interaction of these retinoids with visual cycle enzymes. As a first approach to identifying functional domains and protein recognition sites in CRALBP, a low resolution topological and epitope map has been developed using monoclonal and polyclonal antibodies and limited proteolysis. Fifteen peptides of 8-31 residues spanning 99% of the 316-residue bovine CRALBP were synthesized and used to prepare 13 anti-peptide polyclonal antibodies. Using a competitive ELISA procedure, peptide epitopes were classified as either accessible or inaccessible in the native protein based on the extent of their recognition by these site-specific antibodies. Use of the synthetic peptides to map the epitopes of a polyclonal antibody to intact CRALBP confirmed that the amino terminus and carboxyl terminus are immunodominant regions and hence likely to be exposed, at least in part. Limited tryptic proteolysis of native CRALBP produced three major fragments which were shown by microsequence and Western analysis to be derived from sequential loss of short peptides from the amino terminus. None of these major fragments reacted with four monoclonal antibodies (mAbs) to intact CRALBP although each mAb immunoprecipitated native CRALBP. These results and the lack of mAb recognition of any of the synthetic peptides indicates that the amino terminus of the protein is exposed and contains part of an assembly epitope recognized by the mAbs. Overall this study indicates that residues 1-30, 100-124, and 257-285 contain highly exposed segments in the native protein and therefore constitute potential interaction domains for CRALBP and visual cycle enzymes. Residues 30-99 and 176-229 are inaccessible in the native structure and may be involved with retinoid binding. These results provide a basis for a systematic higher resolution mutagenesis study directed toward correlating CRALBP structural domains with function.

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